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DEPARTMENT OF AGRICULTURE,
NEW SOUTH WALES.

SCIENCE BULLETIN, No. 21.

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THE CAUSE OF BLACK DISEASE AND ITS METHOD OF TRANSMISSION.

BEING FURTHER STUDIES IN A BRAXY-LIKE DISEASE OF SHEEP.

SYDNEY DODD, D.V.Sc., F.R.C.V.S.,
Lecturer in Veterinary Pathology and Bacteriology, Veterinary School, The University of Sydney.

Workers in the respective branches of Economic Science covered by this series of Science Bulletins will receive such of them as may be of use in their special branches of study upon application to the Under Secretary and Director, Department of Agriculture, Sydney.

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The Cause of Black Disease and its Method of Transmission.*

Being Further Studies in a Braxy-like Disease of Sheep.

In a previous article† on black disease in sheep in New South Wales, the writer described the history, symptoms, &c., of this condition, and detailed investigations he had carried out regarding its cause. During this research no positive results such as would justify a careful worker in drawing any definite conclusions, were arrived at, and no definite opinion was expressed as to the cause, although three possible causal factors were discussed, viz., bacterial, nutritional, and plant poisoning. Reasons were given for rejecting the two latter hypotheses. The probable bacterial origin was discussed, and it was stated that the morbid anatomy of the disease indicated a toxaemia, although at that period no bacteria to account for the condition had been demonstrated with sufficient constancy in the tissues, &c., of animals dead from the disease, provided the precaution had been taken to remove such materials immediately after death to reduce the probabilities of agonal invasion to a minimum, and to completely avoid post-mortem invasion. It was also suggested that it was quite possible that the cause was bacteria which were confined to lesions somewhere, or to the intestines, where a toxin was elaborated. Furthermore, it was demonstrated that when exudates, heart blood, portions of organs, &c., taken at random, were removed immediately after death, no bacteria, with certain exceptions, could be cultivated from them.

In the earlier studies, mentioned above, a description of the post-mortem appearance of sheep dead of black disease was given, but this was compiled from autopsies made on animals, many of which had been dead an unknown

* The matter comprising this bulletin has been written some time, but owing to various circumstances a full description of the cultural and other characteristics of the causal organism has not yet been completed. Some of the former are described herein, but these relate mainly to growth in liquid media. It is fully realised that in view of the recent advance in our knowledge of the biology of the anaerobes, no description of an anaerobic organism can be considered complete unless the cultures are started from a single colony, in order to avoid the possibility that one may be dealing with mixed cultures. The writer had determined to withhold publication until this had been done, but circumstances have arisen which render it desirable that the results of six years' research in black disease be made known without any further delay. A complete description of the cultural characteristics of the causal organism, and some notes on serum reactions, &c., can be furnished subsequently. The present matter also appears in the *Journal of Comparative Pathology and Therapeutics.*

† Studies in "Black Disease," *Agricultural Gazette of New South Wales*, vol. xxix, page 657.

although short period. It is thought desirable to record here the appearance of black disease sheep in which secondary changes have not had time to occur. The following is compiled from post-mortem examinations made on over a hundred such cases that have been seen to die, or have been killed, the examination being conducted at once.

The carcase is usually in good condition; and although fluke disease is very prevalent in the black disease localities, I have not seen an animal in the advanced stages of fluke infestation, affected with black disease. This is also the common opinion of sheepowners. The subcutaneous vessels are usually engorged, but not invariably so. No muscular lesions have been seen.

Either the thoracic or the abdominal cavities contain a varying amount of straw-coloured, odourless exudate, usually rather turbid in the case of the latter. A similar clear exudate may at times be found infiltrating the intermuscular tissues of the floor of the abdomen and, rarely, the perirenal tissues also. There are no distinct changes in the lungs. Almost invariably the pericardium is distended with a clear, straw-coloured exudate which soon coagulates on standing. If the examination has been delayed, these exudates may be found more or less blood-stained. The heart frequently shows hæmorrhages in the endocardium of the left ventricle, and at times on the epicardium also. These endocardial hæmorrhages may in some cases, however, be agonal in origin.

The liver, kidneys and spleen are congested, but there is no pronounced swelling of the latter. All the livers examined have shown evidence of recent fluke invasion. This organ almost invariably presents one or more necrotic foci, greyish or dirty yellowish-white in colour, firm and odourless, varying from $\frac{1}{4}$ to 2 inches in diameter, usually circular but occasionally elongated. The edges are irregular, but sharply circumscribed. These foci may be situated anywhere in the liver substance, often superficial, but at times deeply placed and then only free incision will reveal them. Not infrequently, rather circular, hæmorrhagic areas up to an inch in diameter may be met with in the liver also. The abdominal and thoracic lymphatic glands are congested and moderately swollen. The mucosa of the abomasum is frequently congested, often in patches. In the latter case, it is usually most conspicuous around the pylorus. Small hæmorrhages may be scattered over the surface of the organ. At times (but this is by no means common) circular, superficial ulcers may be seen on the mucous membrane of the abomasum. It is probable that these ulcers when they occur are really peptic and of a secondary character. Certain areas of the mucosa are more severely damaged by the bacterial toxin or by hæmorrhage, and the digestive action of the gastric juices acting on this locally injured spot leads to a loss of substance, an ulcer resulting.

The small intestines are more or less deeply congested. Occasionally they are hæmorrhagic, the congestion being most pronounced in the duodenum. At times this is the only part of the bowel affected. The large intestine is

normal. Not infrequently the omentum may present numerous small hæmorrhages scattered throughout its substance. The urinary bladder and urine appear normal.

It must be borne in mind that the foregoing represents the appearances at the time of death. If only a few hours have elapsed between death and the holding of the post-mortem examination, various changes often take place which will present quite a different picture. These have been confounded with the lesions of black disease (just as is the case with the published descriptions of the lesions of braxy in Great Britain) but must be viewed as being of purely post-mortem origin.

With the knowledge gained from the previous researches, investigations were continued during 1917, 1918, and 1919, in the field and the laboratory. It was evident that if the cause were bacterial and capable of being transmitted artificially, it was not resident in any of the materials just mentioned. The nature of some of the experiments showed that it was not a filter passer. The question then, after reviewing the subject and assuming that the cause was bacterial, resolved itself into one of two possibilities, viz. :—

(1) The causal organism was confined to the lesions, whatever these might be.

(2) It was confined solely to the alimentary tract.

Experimental work eliminated the second possibility, since the feeding to a number of sheep of minced up intestines, stomach, &c., produced no positive results, and the ascertained facts in connection with natural black disease did not support the view that infection might be due to minute injuries to the mucous membrane of the intestines. The problem was thus narrowed down to the question as to whether the causal organism, if any, was only to be found in the lesions. As before mentioned, the examination of animals before post-mortem changes had time to set in enabled one to determine which were black disease lesions and which were not. Now, although bacteria could readily be detected in the congested mucosa and submucosa of the abomasum and small intestines when a little time had been allowed to elapse between death and the removal of the tissues, these areas were found upon microscopic examination to be bacteria-free if such tissues were removed and placed in a fixative solution immediately after death. The same could be said of all the lesions in the various parts of the body, with the exception of those portions of liver containing necrotic foci. It was very evident, therefore, that if the bacteria present in such situations, with the exception of the last mentioned, were the cause of death, they must primarily have grown elsewhere, and that the submucosa of the stomach and intestines, the kidneys, blood, &c., had only been invaded during or subsequent to the death agony. Attention was, therefore, more and more focussed upon the necrotic areas in the liver. It will be seen on reference to my previous article, that even when these parts were removed directly death had occurred, there was histological evidence of an inflammatory reaction, and that the necrotic foci contained numbers of bacteria. The opinion was, however, expressed at the

time that these bacteria were probably secondary invaders, since certain hæmorrhagic areas occasionally encountered in the same organ appeared under the microscope to be bacteria-free. At that period it was thought that these hæmorrhagic foci were the precursors of the necrotic areas. Accumulated knowledge has led me to change that opinion, and to now consider that the latter are not secondary to the former, and that the hæmorrhagic foci may even be due to an entirely independent cause.

To follow the reasoning in connection with the cause of black disease, it is desirable to describe more fully than previously the hepatic necrotic areas met with in this condition. One or several of these lesions may be present. Not infrequently they may show no surface indications of their existence, their presence only being demonstrated by free incision. In view of this, it is quite possible that in the early days of the investigations where no necrotic lesions were recorded, such may have been present but escaped detection because not specifically looked for. It should be mentioned, however, that on two occasions where the animal showed clinical and post-mortem signs of black disease (with the exception of these liver lesions) no necrotic areas could be detected after search. This feature will be referred to later and a possible explanation suggested.

The naked eye characters of the necrotic areas have already been described.

Histology of the Necrotic Liver Focus.

Sections show the lesion to present the appearance of coagulative necrosis. It is surrounded by a broad zone of leucocytes, which sharply delimits the normal from the necrotic portions. Immediately within the leucocytic zone are bacilli, often in thick felted masses, arranged around the whole circumference of the lesion. Many of these bacilli are of sufficient length to approach that of the filamentous. Scattered among them are single vegetative elements, but there are no long chains of many individual rods. Occasionally a sporing bacillus with a terminal or subterminal spore may be seen. The bacillary rods are thick, and have rounded ends. If death has elapsed a little before the removal of the affected portion of the liver, numerous bacilli will be found sporing. Scattered throughout the whole of the necrotic area are bacilli having the same morphology—sometimes numerous, at other times scanty. They are, however, usually single, and toward the centre of the lesion filamentous forms are rare. Here and there rounded coccus-like organisms may be seen, but these are merely the bacilli standing on end in the section. The organisms are usually present in the lesions in a state of purity, and they are strictly confined thereto up to the time of death, for although numerous sections from the livers of various affected sheep have been examined, no bacilli could be detected microscopically in the liver tissue outside the leucocytic zone surrounding the area of necrosis, not even in the hæmorrhagic foci, which are occasionally encountered in the same liver. This absence of bacilli from all places except the area of necrosis does not include those very infrequent occasions in which the animal has died from clinical black disease, but there has been no naked eye evidence of focal

necrosis in the liver. Yet bacilli having the characters of black disease bacilli have been isolated from that organ. It will be seen later that it is considered that these cases are ones of mass infection, the animal dying before a gross lesion has had time to develop.

It is quite probable that invasion of the surrounding liver tissue from a necrotic focus and thence of the blood vessels, in conjunction with bacteria from the intestines may take place very soon after death. The histology of the lesion and the disposition of the bacilli recall that of bacterial necrosis of the liver, much more common in cattle, however, than in sheep, due to the necrosis bacillus, although the morphology of the two organisms differs very considerably. In my former article, in order to guard against arriving at conclusions without sufficient evidence, it was stated that the mere presence of organisms in a lesion is not in itself sufficient to prove that those particular organisms are the cause of death of the animal in which they may be found, or that they are the cause of the disease under investigation. When, however, in animals affected with a particular disease, certain lesions are of very common if not invariable occurrence in an organ, and when in such lesions there are constantly found bacilli apparently in pure culture, further investigations are indicated as to the rôle they play. When portions of an organ containing such lesions are removed immediately after death in an aseptic manner, and placed at once in a fixative solution, the probability of *post-mortem* invasion of such tissues by bacteria is negligible. Furthermore, in addition to the necrotic lesions containing the particular organism in large numbers, there is also distinct evidence of reaction on the part of the tissues to the bacterial invasion, and the bacilli in the cases just mentioned, as far as has been ascertained, are confined to these necrotic foci.

All the foregoing factors enumerated, taken collectively, should afford satisfactory evidence that the bacteria in question were present in the situations where they have been demonstrated, some time before death occurred. They, therefore, are clearly not agonal invaders.

The question to be decided is whether the rôle they play in black disease is the primary, or only a secondary one? In my former article, I remarked that they were apparently secondary invaders, because they were absent from the hæmorrhagic hepatic foci which one occasionally met with, and because inoculation experiments on a guinea-pig and a rabbit were negative. It was at that time thought by the writer that these hæmorrhagic areas were the precursors of the necrotic lesions. This, as before remarked, I now consider in all probability to be an error; the former may have quite an independent origin, *e.g.*, early acute fluke infestation, or perhaps they are due to the toxin produced by the causal bacteria. For the failure to infect a rabbit and a guinea-pig with cultures of organisms isolated from a necrotic liver focus in Case 14, described in the before mentioned article, several explanations present themselves; *e.g.*, the bacillus may have become attenuated by various means during the laboratory manipulation, especially by cultivation in glucose media, not much particular attention being paid to the preservation of virulence, as the organism at that time was thought to be a secondary

invader, and with such attenuated organisms the dose administered was too small. That the bacilli do become attenuated in this manner has been demonstrated, even when fresh sub-cultures are employed for inoculation. More detailed experimental work showed pretty conclusively that the bacillus in question could not be regarded merely as a harmless secondary invader of tissues already damaged, for such work demonstrated that small amounts of virulent cultures of the organism are capable of bringing about a speedily fatal result upon par-enteral inoculation, and that the lesions thus artificially produced by it in the sheep are anatomically those of black disease.

It remains to be stated that the foregoing views are not hasty conclusions based upon insufficient evidence, but are the outcome of the examination of, and experimental work with, tissues from a large number of sheep dead of black disease, and as controls, sheep naturally affected with various other conditions.

The Causal Organism of Black Disease.

Morphology.—The organism is one of the larger sporing bacilli. Its length varies considerably, depending on the element on or in which it has been growing. Its breadth is, however, fairly constant. In the natural hepatic lesions its length is from about 4 to 8 microns, with numerous filamentous elements up to 60 microns, without apparent division. The length of the majority of these filamentous forms is, however, about 30 microns. Occasionally chains composed of three or four shorter elements are encountered, but single rods are in the majority. The ends are distinctly rounded. The breadth is about 0·8 to 1 micron. Scattered among the before-described forms may be seen here and there short bacilli, about 2 microns long, and also even what at first sight appear to be coccus-like elements. These latter, as already stated, are merely the ends of the longer rods, standing at right angles in the section. The short rods are the ordinary bacilli lying obliquely to the line of vision in the section, as may be readily demonstrated by altering the focus of the microscope, the rest of the rod then coming into view, whilst the former part disappears. The filamentous elements are almost always to be found arranged around the periphery of the lesion—often in masses—near the leucocytic zone. The bacilli scattered throughout the body of the lesion are of the shorter type.

In cultures the bacilli usually occur singly or in pairs. No chains are formed, and only an occasional filamentous element is seen. In liquid media their length is from about 4 to 7 microns, and their breadth from 0·8 to 1·2 microns. Occasionally rods up to 10 microns occur, but they are seldom less than 4 microns long. In primary cultures from the muscle of inoculated sheep in serum-formate broth, elements up to 17 microns long are frequently seen. Involution forms often occur when the media or surroundings are unfavourable. In smears taken from the local lesion of experimentally infected animals the bacilli vary from 2·5 to 7 microns in length, but their breadth, as in other cases, remains fairly constant, viz., about 1 micron. The longer bacillary forms predominate, but there are no chains or filamentous elements. The organism is non-motile.

Spores.—These are almost invariably situated terminally or sub-terminally, and are distinctly oval, measuring about 1·75 to 2·75 by about 1·25 microns. They distend the bacillary rod. Spores are not often found in the lesions if the latter are fixed directly after death, but they will be found if there has been a few hours delay in fixation. They are readily formed in media containing serum, but very few in any media containing glucose.

Staining reactions.—The bacilli stain with any of the basic aniline dyes and are Gram positive ; but in sections, if the tissue has been preserved any length of time, the contained bacilli cease to retain the stain by the method of Gram or Gram-Weigert, or retain it only feebly. By the Claudius method they stain a deep purple. For the demonstration of the bacteria in sections, the latter has been the method of choice, as the decolorisation of the tissue elements can be carried to a considerable length without decolorising the organisms. In sections also, the Giemsa and Kühne-Nicolle methods have given good results, but more care has to be observed in the manipulation.

Occurrence in the body.—In naturally occurring cases, if the autopsy be held immediately after death, the baccilli will be found confined to the hepatic lesion or lesions. The unaffected liver tissue, other organs, exudates, blood, &c., appear to be quite free from them, even on cultivation. Consequently the condition is really a toxæmia. There is every reason, however, to believe that invasion of the adjoining liver tissue, and possibly the blood, may take place soon after death, together with invasion from the intestines of cadaver bacilli. Invasion by the latter, of course, invariably occurs unless the tissue is removed directly the animal dies. In two cases where the post mortem was unavoidably delayed for about an hour, microscopical examination of the affected liver showed that the bacilli in the necrotic lesion had already begun to extend through the zone of reaction into the unaffected liver substance.

In experimentally infected cases, the disposition of the causal organisms depends upon the method of introduction. When inoculated subcutaneously, the bacilli are found in greatest number in the neighbourhood of the inoculation site. If the injection has been made intramuscularly, they will be found numerously present in the inoculated muscles, but very frequently the subcutaneous exudate immediately in their vicinity may be very scanty in bacilli. Although this exudate may be abundant, and the causal organisms numerous in it around the site of inoculation, it is, nevertheless, difficult by the microscope to find bacilli at any considerable distance from this area, and at times in such cases even cultural methods fail to reveal them. The thoracic, peritoneal, pericardial, and intermuscular exudates (when the latter occurs some distance from the inoculation area) when present in such experimental cases are bacteria free.

Cultural Characters.—This description, as noted in the footnote on page 3, is incomplete, and relates mainly to culture in liquid media. The bacillus is an anærobe, and grows best at body temperature, but, like other anærobes,

can be grown ærobically by adopting Tarozzi's method or one of its modifications. Nissa's liver-piece broth has been found very convenient, with a slight modification as follows:—The minced liver is placed, whilst fresh, in the tubes or flasks, instead of being previously boiled. A sufficient quantity of bouillon is then added, and the tubes, &c., sterilised in the usual manner. The medium, which has coagulated into a solid mass, is broken up by means of a sterile glass rod, and, just prior to inoculation, the tubes are boiled for a short time to drive out the air which gains entrance during the storage of the tubes, the latter being cooled immediately before actual inoculation. Growth in such medium is more abundant than if the liver is cooked before it is placed in the tubes. In this medium the bacillus grows well, spores form readily, and the virulence is maintained for some months without the need for re-inoculation of cultures, provided, of course, that fresh sub-cultures are made when required for animal inoculation.

Serum broth.—Growth is abundant in this medium, and usually complete in from twenty-four to thirty hours. The growth settles at the bottom of the tube in a flocculent mass. Spores are readily formed. In plain broth the growth is very scanty. There is no gas formation.

Glucose-serum broth.—Growth is abundant, but very few spores are formed—and the culture soon dies out. Cultures in media containing glucose soon lose their virulence, and then even large amounts fail to infect. In glucose there is a little gas formation.

Liver-piece broth.—Growth in this medium is abundant, whether under petroleum oil, or exposed to the air, and is complete in twenty-four to thirty-six hours. Numerous spores are formed. The production of gas is considerable. It has a peculiar but not putrefactive odour. The medium is rendered very acid.

Milk.—Grows well. The milk commences to peptonise in twenty-four hours. There is little gas formation. In forty-eight hours the whole of the milk is peptonised, leaving a cloudy, whey-like fluid with loose clot-like deposit. The odour is most unpleasant.

Alkaline egg broth.—Growth is profuse. There is no gas formation. Spores are formed freely. A thick deposit of bacteria forms at the bottom of the tube.

Brain broth.—Grows freely, but the medium is not blackened.

Cultures in liquid media can be readily obtained by floating about half an inch of soft petroleum or liquid petroleum on the surface of the tube before sterilisation. Such, however, does not form a permanent air seal, and the tubes must, therefore, be boiled just prior to inoculation in order to drive out the infiltrated air. Olive oil was not used as an air seal, because it has been found to be quite inefficient for that purpose.

Glucose-agar stab culture.—Growth is evident in less than twenty-four hours along the needle track. No lateral branching is seen. There is a moderate amount of gas formation, and the medium is soon shattered. There is little or no spore formation, and the culture soon dies out.

Experimental.

In the previous article I dealt with the numerous inoculation experiments upon various animals, made during the investigations from 1914 to 1917, with a variety of materials taken from sheep dead from black disease, and also with bacteria isolated from different situations, some of which were pathogenic and others non-pathogenic. Of the former, it was shown that no matter how virulent the organism was on par-enteral inoculation, the value of the conclusions was quite nullified unless it could be shown that the particular organism had been present in the body (in the strict sense of the term) before death. Consequently, deductions based upon experiments made with bacteria isolated from sheep "found dead," particularly anaerobes, could have very little weight.

The present account of experimental work deals with the bacillus considered by the writer to be the causal organism of black disease, isolated in pure culture from various necrotic foci in livers of sheep affected with that condition, the tissue having been removed immediately after the death of the animal. To obtain such tissue, the usual procedure was followed, viz., the particular flock of sheep, usually numbering two or three thousand, in which cases of black disease were occurring, was closely watched and followed about the paddock in which they were grazing. Immediately an animal was observed sick, it was kept under closer observation for a variable period. Usually, however, as soon as it was seen to be seriously ill, it was caught, placed in a vehicle or on a horse, and taken to the spot arranged for conducting the autopsies. Here again the animal was sometimes kept under observation for a variable period, and at other times was killed at once.

During the course of the field investigations, lasting about six years, sheep have been killed in various stages of the disease. One has not always waited until they were *in extremis*; not infrequently the animal has died whilst being transported from the paddock to the post-mortem shed, often a journey of only a few minutes. Yet, although the flock had been closely watched, the animal had only a short time previously been singled out from its fellows as being ill. This difficulty of picking out a sheep before it is seriously ill, from a flock of sheep kept under Australian conditions, has already been commented on by the writer.

Such specimens as were required were taken from the animal immediately it had died naturally or had been killed. Rigid precautions were taken to prevent contamination of the materials during the process. Instruments, test-tubes, specimen bottles, &c., were all previously sterilised by the autoclave in the laboratory before leaving, because in an open paddock, no matter how long one boiled instruments in the field, there might be a possibility that complete sterilisation had not been effected, owing to the vessels in which the sterilisation was conducted being exposed to dust, &c., during boiling. This rendered one's baggage both bulky and heavy, but it had the crowning merit of certainty as regards sterility of instruments, &c.

Selected portions of organs, etc., were placed in test-tubes, wide-mouthed specimen bottles, &c., with the usual precautions, and taken back to the laboratory for further work. Even after the utmost care some of the tubes or bottles were subsequently found to be contaminated, no doubt by air-borne organisms that gained entrance as the specimen was being placed in the receptacle. Any such were rejected. As a rule, with every visit to the affected localities during the season, one could always rely on obtaining sufficient uncontaminated material for subsequent experimental work in the laboratory.

In this experimental work, sheep of any age over nine months were employed. The usual sites of the inoculations were the subcutaneous tissues of the insides of the thighs, the chest and neck; also the muscles of the former. The inside of the thigh was preferred, however, on account of the ease with which one could watch the development of the lesion clinically, it being naturally devoid of wool, and capable of being manipulated post-mortem with least fear of accidental contamination of the exposed tissues. With the rabbit and guinea-pig, the inoculations were made subcutaneously and intramuscularly, usually into the thigh. In the fowl and pigeon the sites were the pectoral muscles.

Young sporing cultures about twenty to twenty-four hours old were usually employed, either in serum-formate broth or liver-piece broth. The cultures from which these inoculation sub-cultures were made might, however, be some months old.

In cases autopsied immediately after death, the bacilli could be recovered in purity from the local lesion and the exudate around it. In the following notes on the experimental inoculations, it may be accepted that unless the contrary is stated, the purity of the organisms from lesions so produced was tested microscopically and culturally, and at times by inoculation into another animal. In quite a number of cases it happened that no matter what care was taken to regulate the dose of culture and the time of inoculation, the animal would be found dead upon one's arrival in the morning. In the case of sheep, although in some instances it was evident that death had not long ensued, post-mortem invasion by other organisms had already taken place, so that bacteriological work on that particular animal was rendered quite nugatory on account of the character of the organisms being experimented with, although the lesions themselves were not masked by post-mortem phenomena. In the case of the smaller animals, those not killed or not seen to die were discarded, and not used in the compilation of experimental data or the drawing of conclusions.

Sheep.

The administration of relatively large amounts of young, virulent, sporing cultures by the mouth has quite failed to produce any evidence of reaction, although a number of methods were employed to facilitate the action of the organism and to prevent its destruction by the digestive agencies of the animal. This is quite in conformity with the feeding experiments made with minced organs, viscera, &c., of naturally affected sheep, as previously

recorded. Subsequent inoculation experiments with sheep that had been drenched with cultures, indicate that the administration of virulent material by way of the alimentary tract confers little or no immunity.

Subcutaneous and Intramuscular Inoculation.—Small doses up to 0·25 c.c. produce in the sheep considerable local and systemic disturbance, but are not always fatal. The latter amount will frequently kill, but the result is not constant. Doses of from 0·5 to 1·5 c.c. of culture are almost invariably fatal to sheep of any age in from about twenty-eight to seventy-eight hours, usually in less than forty-eight hours. Sheep that survive experimental infection for three days often recover. With subcutaneous inoculation there is, about fifteen hours afterwards, considerable swelling around the site of injection, which gradually extends, at first mainly in a downward direction (gravitation). If the injection be into a limb, the swelling gradually reaches the coronet. As the condition progresses pronounced lameness appears, the animal often carrying the affected leg. The swollen part in the early stage of the disease is œdematous, and pits readily on pressure ; later on it becomes firmer. There is no noticeable gas formation. For a while the skin itself shows no sign of change, but about twenty-four hours after the injection a small livid spot appears at the point of entrance of the needle. The lividity gradually spreads, its actual extent appearing to depend upon how long the animal survives. If death occurs in about forty hours, it may be only about half an inch or so in diameter. If the animal lives longer, the area may extend several inches around the needle puncture. In cases where a slightly attenuated culture (artificially attenuated) has been employed, and the animal has survived, a very pronounced livid area of varying extent appears at the inoculation site, succeeded by necrosis of the overlying skin.

There is a moderate hyperthermia (about 105 deg. Fah.), with the usual clinical manifestations of profound systemic disturbance. Later, the animal becomes very quiet, and readily permits itself to be handled, or may even refuse to move. At this period, however, the mental faculties do not appear to be impaired. In the final stages the animal becomes comatose and dies very quietly.

Post-mortem Appearances.—If the autopsy has been made immediately after death, the most striking changes are observable in the inoculated area. In the case of injection into the thigh, the wool is easily pulled out of that limb. An œdematous swelling extends downwards to the coronet and upwards to the groin. At times, however, the upward extension of the swelling is absent. At others, the upward extension, instead of gradually diminishing, terminates abruptly. Possibly this is the effect of gravity on the contained exudate.

As already remarked, there is a larger or smaller area of lividity around the point of inoculation. If death has ensued soon after the injection, this area may be minute. If the animal has survived two days or so, the subcutaneous tissue in the inoculation area is considerably thickened, and shows evidence of necrosis. The subcutaneous tissues of the swollen part are saturated with an odourless exudate, which, near the inoculation site, is often

blood-tinged, but elsewhere is clear and straw-coloured. At times this exudate may be found reaching to the floor of the abdomen, and even infiltrating the inter-muscular tissues of the abdominal muscles. On a few occasions the perirenal tissue was also found saturated with this exudate. There is no evidence of gas formation.

Bacilli in purity may be found fairly numerously around the site of inoculation, but they are extremely scanty in the subcutaneous exudate at any distance from this area, and their presence there often can only be demonstrated by cultivation. At times even this fails, especially in the case of the exudate in the abdominal floor and other such distant sites. If the injection has been made intramuscularly, the reaction is more severe, although at times the swelling of the limb is not so pronounced. In addition to the phenomena mentioned as resulting from subcutaneous inoculation, the muscles around the inoculation site are very dark and may show a number of hæmorrhages. On incision of the affected muscles there is a distinctly unpleasant odour, but not exactly that of putrefaction. There is no evident gas formation. Parts of the muscle appear at times to have a greyish necrotic character. Others in the immediate vicinity may show a few small hæmorrhages scattered throughout their substance. The affected muscles are infiltrated with a blood-tinged, odourless exudate.

Occasionally there may be some clear exudate in the peritoneal or pleural cavities, but as a rule there is none. Almost invariably, however, the pericardium is distended with a clear, straw-coloured odourless fluid, which, like other exudates, coagulates spontaneously. It is bacteria free. If the heart, blood and organs are examined immediately after death they also are found free from bacteria. Frequently the heart shows endocardial hæmorrhages. The liver, kidneys and spleen are congested, but not enlarged. Sometimes, but not often, one may see on the surface of the former dirty, greyish-white areas of commencing necrosis, with ill-defined margins. Usually there is some more or less pronounced œdema and congestion of the mucosa of the abomasum and small intestines, but no ulceration. The lymphatic glands of the inoculated limb, and of the trunk also, are congested and swollen. There are no naked eye changes in the lungs.

Guinea-pigs.

With virulent, young cultures, doses of 0·1 c.c. injected subcutaneously are usually fatal in about twenty hours. With smaller doses of 0·02 to 0·05 c.c., the animal may live for about three days. In all cases, however, the lesions are fairly constant, viz., considerable swelling of the inoculated thigh, the skin being tense and sometimes livid. The subcutaneous tissues of the inoculated leg are saturated with a slightly turbid, odourless exudate. There is no gas formation. The subcutaneous exudation extends forward along the abdomen for some considerable distance, often as far as the axillæ. It is more abundant on the inoculated side of the body. Around the point of injection, the subcutaneous tissues show varying degrees of thickening, according to the duration of the disease. The muscles in the same area have

in parts a greyish appearance of commencing necrosis, and in others a pronounced inflammatory congestion. They are not often actually hæmorrhagic. On section, if the injection has been intramuscular, the injected muscles have an unpleasant but hardly putrefactive odour. Occasionally, there may be a slight amount of exudate in the peritoneal or pleural cavities, but this is not the rule. The spleen, kidneys and liver are congested, and if the disease has lasted twenty-four hours or more there are very occasionally either ill-defined or circumscribed dirty-white areas of commencing necrosis in the latter, about an eighth of an inch in diameter. The stomach and small intestines are as a rule deeply congested, but there is no ulceration. The mucosa is œdematous, and the contents of the small intestines usually a straw-coloured, clear fluid The pericardium is almost invariably distended with a clear, colourless exudate. As a rule the causal bacteria are confined to the neighbourhood of the site of the inoculation and the subcutaneous exudate of that region, from which they can be recovered in purity, but the further the exudate is from the local lesion, the more scanty become the bacilli.

Rabbits.

Small doses of from 0·1 to 0·2 c.c., such as would be sufficient to kill a guinea-pig are not fatal. Very frequently no reaction at all is to be seen. Larger doses, viz., 0·25 to 0·5 c.c. subcutaneously, usually result in death In a number of instances, even with such increased amounts, death may be delayed until the fortieth hour after injection. Beyond pronounced lameness, there are few clinical symptoms to be observed until near the fatal termination.

Lesions.—There is practically no swelling round the point of inoculation, no cutaneous changes and no gas formation. The subcutaneous tissues in the inoculation area are moderately infiltrated with an exudate, slightly blood-stained near the point of injection, but colourless elsewhere. There is no odour. The superficial muscles in this region are slightly reddened, but there are no hæmorrhages. Frequently there is a considerable amount of exudate in the pericardium but no gross lesions elsewhere. The bacilli can be recovered in purity from the inoculation area, but they are not obtainable elsewhere.

The Fowl.

Doses of 0·2 c.c. of virulent culture injected intramuscularly are usually fatal, but death does not occur under about forty hours or longer. Here, again, there are few clinical signs of reaction; the bird looking apparently well until near the end. Death ensues rapidly and quietly once symptoms are manifest.

Lesions.—There are no marked cutaneous changes. The subcutaneous tissues for some distance around the point of inoculation are saturated with the usual inflammatory exudate. Near the point of entrance of the needle, it is slightly blood-tinged, but some distance away, colourless. These tissues also show evidence of necrosis. There is, however, no unusual odour and no gas. The muscles in the inoculated region and for some distance around, have a greyish-white appearance of commencing necrosis, most pronounced

along the needle track. There is no unusual odour in the incised affected muscles and no hæmorrhages. No other gross lesions have been observed save moderate congestion of the mucosa of the small intestines. The bacilli are recovered in purity from the inoculation area, but elsewhere is bacteria free.

The Pigeon.

This bird is as susceptible to artificial infection as the guinea-pig. The intramuscular injection of 0·1 c.c. of virulent culture is usually followed by death in about eighteen to twenty hours. On autopsy no cutaneous changes are evident. The subcutaneous tissues of the breast and abdomen are infiltrated with the usual exudate. There is no odour or gas formation. A distinctly necrotic line follows the needle track and extends laterally for a short distance into the muscles, the lateral extension being irregular in width. At the edges of this necrotic zone the muscles show evidence of intense inflammatory congestion, but there are no distinct hæmorrhages and no gas. On incision, the odour of the affected muscles is rather unpleasant but not distinctly putrefactive. No other gross lesions have been observed. The bacilli are confined to the lesions from which they have been recovered in purity.

Up to the present, no experimental inoculations have been made into the horse, ox or pig.

The Natural Method of Infection.

Before entering upon a discussion as to the natural method of infection in black disease, it may first be advisable to note some points that have been observed clinically and experimentally in connection with this condition, most of which are already recorded in this and my previous article.

Clinical.—The disease is seasonal, the vast majority of cases occurring from late summer to early winter. In mild seasons occasional deaths will take place throughout the winter. On infected properties, sheep grazing in paddocks well watered by springs but in which the ground is not well drained or which contain swampy areas, not necessarily of very large extent, are subject to a heavy annual mortality from black disease, except in seasons of unusual dryness when the moist places have dried up, whereas sheep kept in paddocks having water from the same sources running through them, but where the ground is well drained naturally, show few losses or none at all. The division between the infected and non-infected paddocks may be merely a wire fence or a stone wall. In infected paddocks that have been artificially drained the mortality has been considerably diminished. In a very dry season, such as that experienced in 1918-19, the death-rate in paddocks known to be heavily infected was unusually light.

There is no evidence of any kind to show that the disease can be communicated directly from one animal to another. It is stated that after the transfer of the whole of an affected flock to a clean paddock a few cases occur for a short time, and then the mortality ceases. These are apparently cases of infection, or rather (as will be shown) of potential infection

before removal. Owners have informed me that no cases occur in a subsequent season in a clean paddock (that is, paddocks in which black disease has never been known to occur) to which an affected flock has been removed, but of this latter I have no direct evidence. All the evidence goes to show that the causal organism of black disease is a facultative parasite, and that the places most dangerous for sheep are the springs and moist, undrained areas in the paddocks. The disease appears peculiar to the sheep, for other animals grazing over the same country remain unaffected. Popular opinion considers that the springs and moist places are the localities whence the disease arises, although the view is that it is due to eating plants growing therein, *e.g.,* watercress. Fluke disease of the liver is very common among all sheep in the area in which black disease is known to occur. Some sheepowners have observed the coincident occurrence of black disease and fluke infestation. This has led a few of them to express the opinion that the former is nothing else but acute fluke disease of the liver. That this is not the case is shown by the fact that fluke disease is common in numerous parts of New South Wales and elsewhere, but black disease or anything resembling it is unknown in many such places.

Experimental.—It has been demonstrated that the administration of large amounts of virulent sporing bacilli by the alimentary tract fails to set up any sign of infection. Consequently the mere ingestion of contaminated water or food can hardly be considered to be the usual method of natural infection. The same deduction may be drawn from the feeding experiments made with minced organs and viscera, &c., from animals dead from natural infection. The subcutaneous inoculation of small amounts of culture are very fatal to susceptible animals, and always gives rise to definite changes at and around the site of inoculation. Mere scarification is insufficient to infect. Seeing that in naturally-occurring cases no cutaneous or muscular lesion is encountered in animals examined immediately they are dead (if one excepts the occasional presence of a clear intermuscular or subcutaneous exudate in certain situations), it is quite reasonable to conclude that the common natural method of infection is not through the skin. The same reasoning may be advanced against the view that the bacilli gain entrance to the body by means of small abrasions of the mucosa of the alimentary tract, including the abomasum. Infection by inhalation may be dismissed as the least probable method of any.

Another important feature of the disease is that of the hepatic lesion or lesions. If this is, as I consider it to be, the primary lesion in black disease, the question at once arises, how does it originate? The writer has made post-mortem examinations on many hundreds of sheep at various times and places, but has not seen these particular hepatic lesions in any condition other than black disease, although focal necrosis of the liver may be caused by other agents. Seeing that experimentally-ingested bacilli fail to infect, one naturally seeks an explanation as to the means whereby the bacilli reach the liver and produce the special lesions therein. After

weighing the arguments presenting themselves, I have arrived at the conclusion that the solution of the problem of the natural method of infection and the situation of the primary lesion in the liver is to be found in the liver fluke. If the feasibility of such a hypothesis be accepted, then almost all of the features concerning the transmission of black disease become fairly clear.

The factors that have been found to coincide with the prevalence of black disease are just those which prevail in connection with the presence of recent fluke infestation. Drainage of marshy areas around and along the course of springs and watercourses, where carried out effectively, has often been succeeded the following season by a greatly diminished mortality from black disease. In fact, before I had demonstrated the cause of the disease, I consistently recommended this plan. When owners now seek advice regarding this condition I generally inform them that if they get rid of the liver fluke they need not trouble very much about black disease. This advice, when followed out by draining the infected places, has at times had a not unexpected result, because paddocks where it was almost certain one could get material in the shape of affected sheep during the season for research work, have now become useless for investigations, as few cases are seen in them, and these at only irregular periods.

Drainage, as is well known, assists in keeping down fluke disease by decreasing the number of water snails (the intermediate host of the fluke), and providing an unfavourable nidus for the fluke embryos, &c. An instance of the foregoing may be cited. A certain paddock running about 2,000 sheep has been notorious for black disease for the past twenty-six years, the average annual loss in it from this condition being about 25 to 30 per cent. The paddock contains several springs and the ground in their neighbourhood was swampy. Up to the end of 1918 fluke disease in this paddock was very prevalent. At the end of that year the paddock was well drained. The season of 1918-19 was very dry. During that year the paddock carried about 1,000 sheep. The mortality from black disease was about 3 per cent. In another paddock, running about 2,000 to 3,000 sheep, where also the previous annual losses from this disease had been considerable, the same conditions obtained in 1918-19 as on the area just mentioned, viz., drainage of the swampy spots and dry season. The percentage mortality from black disease was lowered to about the same as in the previous instance. In both cases the sheep during that season had been under closer observation than usual.

Seeing that the causal organism is a sporing, and in every probability a facultative, parasite, it is not probable that it could have died out in these paddocks in such a short period. The explanation appears rather to be that the intermediate host of the liver fluke, viz., the water snail, had become considerably reduced in numbers, or had migrated to more congenial localities not grazed over by the sheep, and also that many of the fluke embryos, not finding their host, had also died, the consequence being less fluke invasion of the liver of the sheep in these paddocks and less black disease.

In connecting the fluke with the transmission of black disease it is noteworthy that in all undoubted cases of that condition there has been fluke infestation of the liver, and the invasion has been recent; in some cases, however, it was only slight. The animals have all been in good condition. I have never seen a case of black disease in which the fluke disease was very advanced. This indicates that infection occurs very shortly after the carrier fluke gains admission to the liver, and agrees with experimental evidence, the latter showing that the condition is not a chronic one and that the period of incubation is not a matter of several days. In natural cases the period of incubation, of course, would not commence until the bacilli had been lodged in the liver. A greater or fewer number of flukes may reach the liver prior to the advent of the carrier, for it is not suggested that every fluke is an actual carrier of infection, although it is a potential one. This is shown by the fact that a large percentage of sheep on infected country, dead from causes other than black disease, have fluke-infested livers. As a matter of fact, it is possible that relatively few of the total number of fluke parasites reaching the liver carry with them the causal organism of black disease, seeing that the necrotic foci in the liver, which are the primary lesions, are usually so few in number. There may be only one such lesion. In view of the relatively small number of bacilli required to infect artificially, the great number of those present in a single hepatic lesion would be quite adequate to account for the symptoms and death in naturally-infected cases. In the few instances where careful search has failed to reveal a definite local lesion in the liver, but where bacteriological or experimental investigation has shown the bacteria to be present in that organ, it is suggested that such are cases of mass infection, the defences of the organ and the body in general having been overwhelmed before any reaction—that is, a local lesion—has had time to become visible to the naked eye. In the early days of the investigations (1914-15) the liver was not specially examined by making numerous incisions for these necrotic foci, and no doubt those situated in the depths of the organ escaped observation during the usual routine post-mortem examination. When, however, it was found that the absence of these particular lesions was more unusual than their presence, special attention was paid to the liver.

In connection with fluke invasion itself, it has been noted by other observers that bacteria (for example, *B. coli*) have been mechanically conveyed to the liver from the intestinal tract by that parasite.

In discussing the role of the liver fluke in the transmission of black disease, there is no doubt that the causal organism of the latter is numerously present in certain paddocks, probably scattered over the whole surface by dead black-disease sheep allowed to rot where they die and other agencies, but, being a facultative parasite, one would reasonably expect it to be more abundant in the situations more favourable to its saprophytic mode of existence, viz., in moist localities. It may be accepted that sheep in such paddocks will swallow varying amounts of the bacilli in question with their food and water, although such animals drink little

water when food is succulent. Yet, as has been experimentally demonstrated, the mere ingestion of large amounts of virulent bacilli is insufficient to infect. As already noted, *Fasciola hepaticum* is very prevalent in the affected paddocks. Whether the immature fluke (cercaria) becomes contaminated whilst on the ground, or whether it becomes so during its passage through the anterior part of the alimentary tract, is a matter for speculation, but there is no improbability in the latter.

A feature of the experimentally-induced disease is that if minimal lethal doses of bacilli be injected, its course may be prolonged a day or so, and the lesion at the point of inoculation is more pronounced. One can thus understand that if the number of bacilli introduced into the liver by the fluke is insufficient to overwhelm the defences of that organ, there is time for a reaction on the part of the latter to occur, and for a definite local lesion to become evident to the naked eye. That such a reaction on the part of liver tissue does take place as a rule is evidenced by histological examination. The result is that the bacilli are confined to the lesion and no systemic invasion ensues, the general symptoms and lesions elsewhere no doubt being due to an endo-toxin.

The seasonal occurrence of black disease may be explained by taking into account the habits of sheep and the life history of the liver fluke. Fluke infestation of animals is generally considered to occur mainly in spring and autumn, although it may take place at any time. In the spring there is little need for sheep as a whole to graze over the marshy places, which are relatively small in area, in an infected paddock, as the animals drink little water then, and the herbage elsewhere is, in general, succulent. In spring and summer, therefore, the probability of the causal organism being conveyed to the liver by the immigrating flukes is not so pronounced. The disease first becomes noticeable in summer. At first, the death-rate is light, but as the season advances the mortality gradually increases until in late autumn it reaches its height, coinciding with the period at which the migration of flukes to the liver is also at its maximum. The onset of severe frost is generally considered to put a stop to the ravages of the disease for that season, but if the weather be mild, deaths take place throughout the winter. In some places where the winters are not so severe it is stated that black disease may be seen at any time of the year. This latter statement, however, is not to be accepted without reservation, for sometimes any death of sheep is ascribed to black disease merely because the animals have been found dead, no attempts at post-mortem examination having been made. Furthermore, I have known men confidently say that they have known sheep to die from black disease in their district, although they will admit that they have never examined a case of that disease.

In the districts where the disease in enzoötic, the cessation or continuance of infection may be explained if one considers the effects of frosts on fluke embryos and cercariæ. Numbers of the former (not all, by any means) will be killed off, thus lessening the number of cercariæ to be swallowed by the sheep, and consequently the chances of infection by the black disease

bacillus also, until next season. Furthermore, provided there is food elsewhere, there is no inducement for sheep to frequent wet places in the winter.

It hardly needs stating that if the causal organism is not present either contaminating the soil, or mixed with the stomach contents of the sheep, no amount of fluke invasion will produce black disease.

Another interesting feature—perhaps unimportant in itself, but forming one of the links in the chain of evidence—is that for several years past the seasons in general have been on the whole dry, and several large sheep-owners have informed me that during the past few years black disease has by no means has been so prevalent on their stations as in former years.

Although it may be admitted that the evidence put forward to incriminate the liver fluke in its migration from the exterior of the body to the liver of the infected sheep, as being the mechanical agent in the transmission of the causal organism of black disease is largely circumstantial, yet this explanation of the natural method of infection and the situation of the primary lesion or lesions appears more satisfactory than any other advanced so far.

Immunisation.

After the determination of the causal agent it was a natural sequence to endeavour to devise a method of prevention against black disease. Curative treatment, in view of the acuteness of the disease and the conditions under which sheep are kept in Australia, is impracticable. One aspect of prevention has already been dealt with, viz., the rendering of the moist areas unsuitable breeding-grounds for the intermediate host of the fluke (the water snail) by drainage. If this is not practicable such areas could be fenced off. The question of preventive vaccination has been considered, and an attenuated vaccine prepared. It was first tried on small animals, then on sheep, at the laboratory. About sixty of the latter, in batches of three or four, were vaccinated at various periods, and apparently the immunisation was efficacious, as the animals subsequently resisted fatal amounts of virulent bacilli. No reaction to the latter appeared at times, and a moderate local tumifaction developed at others, if a large dose (for example, 3-5 c.cs. of virulent culture) were injected. There were no deaths.

This success determined me to try the value of preventive vaccination in the field. During the seasons 1918-19 and 1919-20 between 5,000 and 6,000 sheep were vaccinated (about 2,600 each season), at such a period as would permit the immunity to be fully established before the onset of black disease. The animals were placed in two notoriously infected paddocks where the annual loss for years had been from 20 to 30 per cent., and several hundred unvaccinated sheep of the same age, sex, &c., were placed with them as controls. The results have been not altogether expected. If the vaccination were unsuccessful one would expect both vaccinated and control sheep to die in the same proportion, whereas if it were successful,

only the control sheep should die in any number. As a matter of fact, during both seasons the mortality in these paddocks from all causes was not more than 2·5 per cent., and there was the same low percentage of deaths in the controls as in the vaccinated animals. As remarked, this tremendous reduction of the average mortality from black disease was not expected to be seen in the unprotected as well as the protected animals, and although the owner (who informed me that he had known the particular station for twenty-six years and in one of the experimental paddocks the mortality averaged 25 to 30 per cent. each season), was satisfied so far as losses were concerned, it did not solve the question as to whether the vaccine was efficacious in the prevention of black disease. I think the real explanation of the great drop in the incidence of the disease in the particular paddocks is that the owner during the summer preceding the vaccination, had thoroughly drained the swampy places, so that the water from the springs could readily get away, and consequently the carriers of the disease had disappeared to a great extent.

Experimental vaccination can be carried out in the laboratory at any time of the year, but the real test of the value of such work lies in its application in the field on a large scale, but owing to the seasonal character of black disease, this can only be carried out once a year, because no matter when the immunisation was made, one would have to wait until the end of the black disease season before any conclusions could be drawn as to its efficacy. If it were unsuccessful for any reason, then one would have to wait another year before undertaking a fresh series of field experiments on a large scale. It follows that such work cannot be accomplished quickly.

The research work in general has been prolonged for a variety of reasons; the strictly seasonal character of the disease (February to May being the usual period), the distance from Sydney (more than 300 miles to the locality of the disease), and the writer being engaged in teaching duties as well as carrying out bacteriological and pathological work for the State at the same time. Perhaps what has handicapped one most of all is the total absence of skilled technical assistance, all work, whether in the laboratory or the field, having in this respect to be carried out unaided. Those who have had to do with the preparation of culture media, cultures, sections, &c., will appreciate what this means. Finally, in view of the pitfalls which other workers have fallen into, it was very desirable that one should take more than usual precautions in order not to make the same or other errors.

The identity or non-identity of Black Disease with the Braxy-like disease of Sheep in Victoria and Tasmania, and Braxy or Bradsot.

It has not yet been established that the above diseases are identical. Several statements in the affirmative have been made, but these are merely opinions, and a definite decision as to whether the diseases are the same or not can only be arrived at by a comparison of the causal organisms, when

the latter have been conclusively proved to be the etiological factor of each condition. In the meantime, however, one may express an opinion as to the probabilities, based on one's own experience and on the published work of others.

The evidence for the identity of black disease and the braxy-like disease in Tasmania and Victoria is very strong, save that the organism claimed by Gilruth to be the causal factor of the latter, is not the same as that responsible for black disease. On referring to his account of his investigations* one finds the following remarks *re* the disease in Victoria (page 570): "The soil is not rich . . . it is fairly watered by means of springs . . . which generally result in the formation of a small swampy area around each. As a consequence, fluke disease is common." Concerning post-mortem appearances he notes among other changes (page 571) "The stomach is always more or less deeply congested, but I have observed no ulceration. *The liver often presents a mottled appearance, due to circumscribed irregular areas of necrosis.*"

As regards the pathogenic organism, he describes what he considers to be the characteristic organism as being present in the effusions, organs, and even in the blood itself, but never in a state of purity. He further adds (page 572): "*The necrosed liver areas also show the characteristic bacilli in great numbers.*"

Regarding the disease in Tasmania, he says (page 570): "The liver is very often congested, *and may show yellowish necrotic areas.*"

The italics in the foregoing quotations are mine.

It is evident that the necrotic hepatic lesions were looked upon as secondary in character, for no experimental work appears to have been done with them. The materials for animal inoculation being blood and exudates, it follows that the bacilli he saw in sections of hepatic necrotic lesions were not necessarily identical with those obtained from the blood and exudates of the same animal or animals, in view of my own experiences.

The indications are pretty clear that black disease in New South Wales and the braxy-like disease in Victoria and Tasmania are identical. Now, seeing that Gilruth definitely claimed to have isolated the causal organism of the latter disease, it is evident that it should be identical with that isolated from black disease. I have had opportunity of examining two different lots of material containing the bacilli isolated by Gilruth, and find that they are not the same, either morphologically or culturally, as those isolated from cases of black disease. This being the case, one has to explain the difference. Reference to my first article will show that in the early period of the investigations my results agreed with those of Gilruth bacteriologically, but it was subsequently shown that the bacilli that had been isolated from sheep found dead, although death had only recently taken place, could not be acknowledged the indisputable cause of the disease.

* Proceedings of the Australasian Association for the Advancement of Science, 1911, vol. 13.

Later on it was shown that they were agonal or post-mortem invaders, as proved by their absence from blood, tissues, &c., when such were removed immediately the animal had been *seen to die*. On reading Gilruth's articles one cannot avoid the conclusion that the same thing happened in the case of the Victorian and Tasmanian investigations, viz., that the bacteria claimed to be the causal organisms were obtained from animals from which the possibilities of post-mortem invasion could not be excluded.

Regarding the identity of the Australian disease with European braxy or bradsot, the matter is more difficult to approach. The bacteriological findings of a number of writers afford little aid in the solution of the question. Some were undoubtedly dealing with post-mortem invaders, whilst others had not eliminated that possibility. As regards the disease seen on the continent of Europe, there is still dispute as to the etiological factor. Here again some of the writers were undoubtedly working with cadaver bacilli. It would greatly simplify matters if it could be shown that whatever bacilli were isolated, they were obtained from animals immediately they were dead, and that means were taken to prevent any secondary contamination of the fluids or tissues, this being an important consideration. In spite of these uncertainties it would appear that braxy or bradsot has a number of features in common as regards climatology and morbid anatomy, if one disregards the obvious post-mortem phenomena in connection with the latter; but (and this I consider to be an important difference) I have not been able to discover any reference to the presence of liver lesions in descriptions of the disease either in Great Britain or on the Continent. If these are really absent, then one must conclude that either the disease is not identical with that seen in Australia, or that the port of entrance of the bacilli is not the same, and that the carrier can therefore hardly be the liver fluke. On the other hand it may be that the liver lesion or lesions have been overlooked by those making the examination, or they have been viewed as merely of secondary importance, and attention has been directed elsewhere, as, for example, to the abomasum. It will be evident that microscopical examination of, or cultures made from, any portion of the liver, save from the necrotic foci themselves, would be entirely negative if means were taken to avoid agonal or post-mortem invasion. The fact that a bacillus, which has been isolated from blood, exudates, or organs of sheep found dead, is very fatal to experimental animals upon parenteral inoculation, is no proof that it was the causal agent of the disease, because it is well known that quite a number of anaerobic cadaver bacilli are highly pathogenic when so injected.

It is rather strange that, although investigators have known of the fallacies introduced by working with cadaver bacilli, yet until the writer pointed it out, no one working on braxy or its allies appears to have realised the primary importance of the fact that in research work on diseases of this nature in sheep, one should always be assured that the organisms isolated were present in the body (in the strict sense of the word) before death, unless it can be shown that invasion of the body itself is not necessary for the organism to produce its pathogenic effects. Of course, it has to be

admitted at once that if the fluke is capable of acting as a mechanical carrier of one species of bacterium to the liver, there is no reason why other species may not be so transmitted, given the opportunity. The writer therefore hopes that this aspect of the method of infection in the type of disease discussed in the present article may be given consideration by workers on braxy and bradsot, and also in the condition met with in various parts of Australia and elsewhere, in order that the writer's hypothesis may in their particular cases be either established or disproved.

SUMMARY.

Black disease is a condition apparently peculiar to sheep, and seasonal in occurrence.

It is a toxæmia, running an acute course. The primary lesions are situated in the liver, and consist of circumscribed areas of necrosis. One or several lesions may be present.

Other lesions seen elsewhere must be looked upon as secondary and probably due to the action of the toxin produced by the bacilli in the primary lesion and distributed in the blood stream.

The primary hepatic lesions contain the causal organism, which at the time of death are confined thereto and cannot be demonstrated elsewhere even in the liver, save for a few exceptional occasions in which no gross necrotic foci were seen but the bacilli were found to be present in that organ. Such cases may be looked upon as ones of mass infection, the defences of the organ having been overwhelmed before they could come into action.

The causal organism is one of the larger anaerobic bacilli, which sporulates readily, and is probably a facultative parasite.

Experimental inoculation of virulent cultures is fatal to sheep and other animals, and the lesions are anatomically those of black disease, the apparent difference being due to the port of entrance of the bacilli and the nature of the tissues there. (Compare contagious bovine pleuro-pneumonia.)

The situation of the primary lesion or lesions, and the failure to infect by feeding experiments with virulent cultures or viscera of affected animals, suggests the probability of a carrier, and the seasonal character of the disease, the geographical features of infected paddocks and the presence of fluke disease, indicates that the liver fluke is the mechanical carrier.

Black disease is probably identical with the braxy-like diseases in Tasmania and Victoria, although the organism claimed to be the cause of the latter is not identical with that isolated from the former. Until more definite information is obtainable regarding braxy or bradsot, in which the possibility of post-mortem invasion has been eliminated, it is not possible to decide whether the European disease is the same as that seen in Australia. It is, however, quite probable that it is.

[The accompanying photographs are by Mr. R. Grant, Bureau of Microbiology.]

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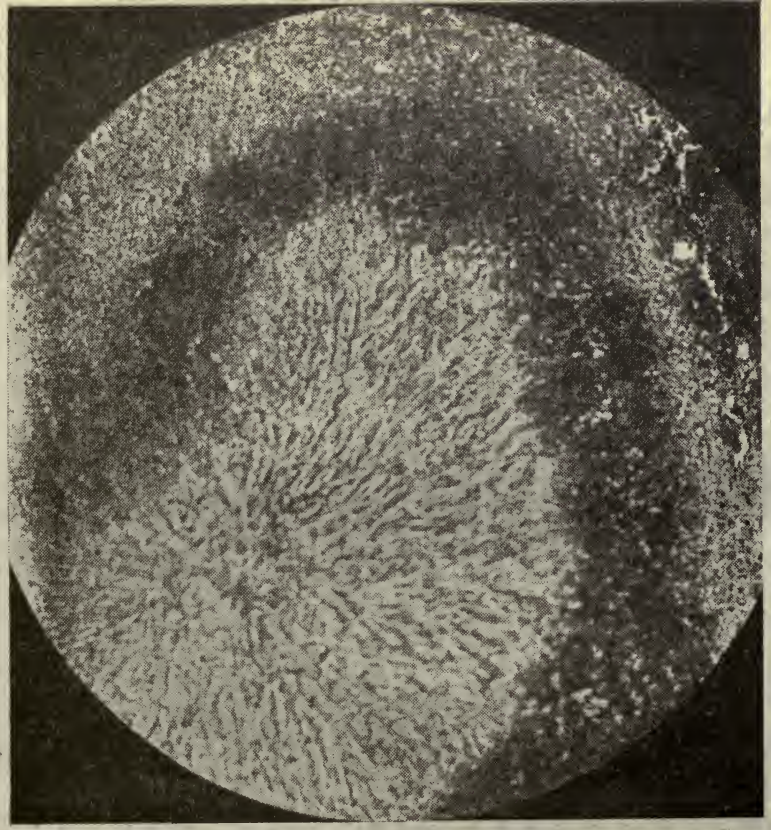

Fig. 1.

Section of liver lesion, from Case 53, stained hæmatoxylin and eosin; showing central necrotic area surrounded by dense zone of leucocytes; outside this is normal liver tissue. Ob: 16 m.m. Oc: 2. 55 c.m. x 145.

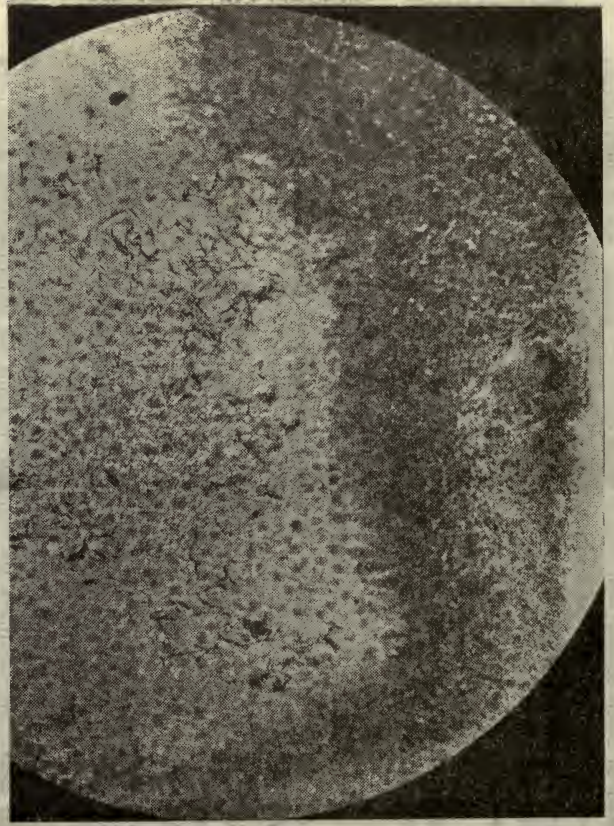

Fig. 2.

Section of necrotic liver lesion, Case 52, stained Claudius and hæmatoxylin; showing large necrotic area, with masses of bacilli grouped around the periphery and individual bacilli scattered throughout the body of the lesion; also zone of leucocytes, and immediately beyond that normal liver tissue. Obj: 16 m.m. Oc: 2. 55 c.m. x 145.

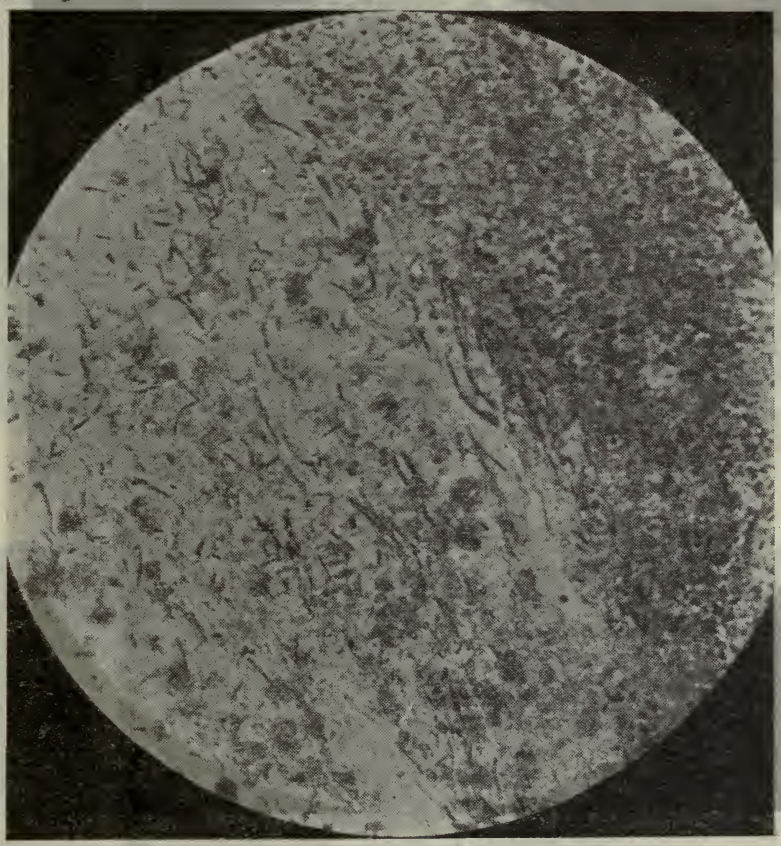

Fig. 3.

Section liver lesion, Case 53, stained claudius; showing necrotic area and zone of leucocytes, the bacilli being grouped in masses around the periphery, and also scattered throughout the necrotic portion. Obj: 6 m.m. No. 2 Oc: 55 c.m. x 400.

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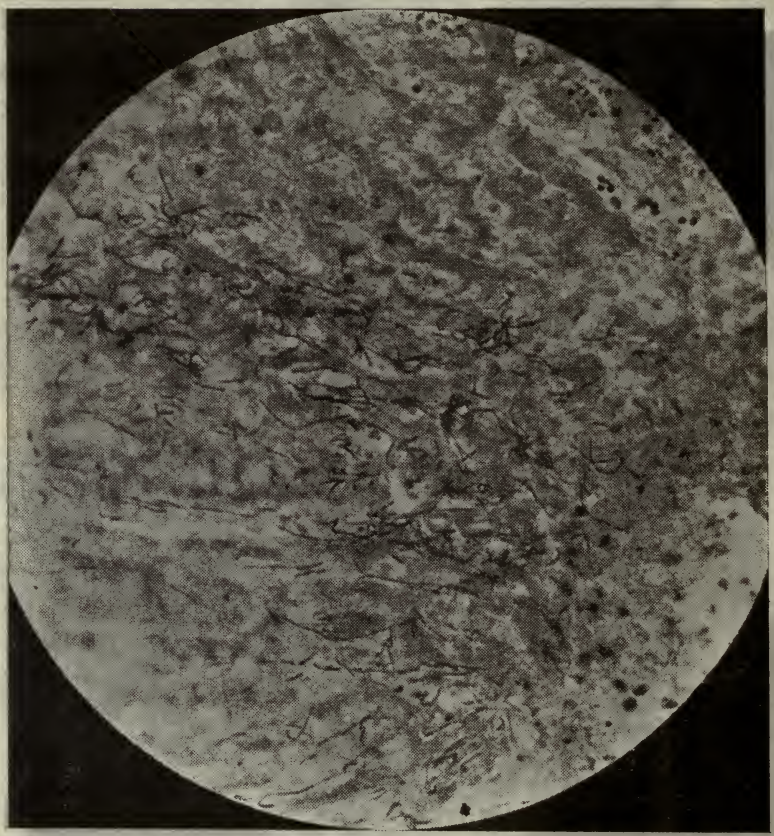

Fig. 4.

Portion of necrotic area in hepatic lesion, Case 52 ; showing the filamentous character of the massed bacilli ; claudius stain. Obj : 6 m.m. Oc : 2. 55 c.m. x 400.

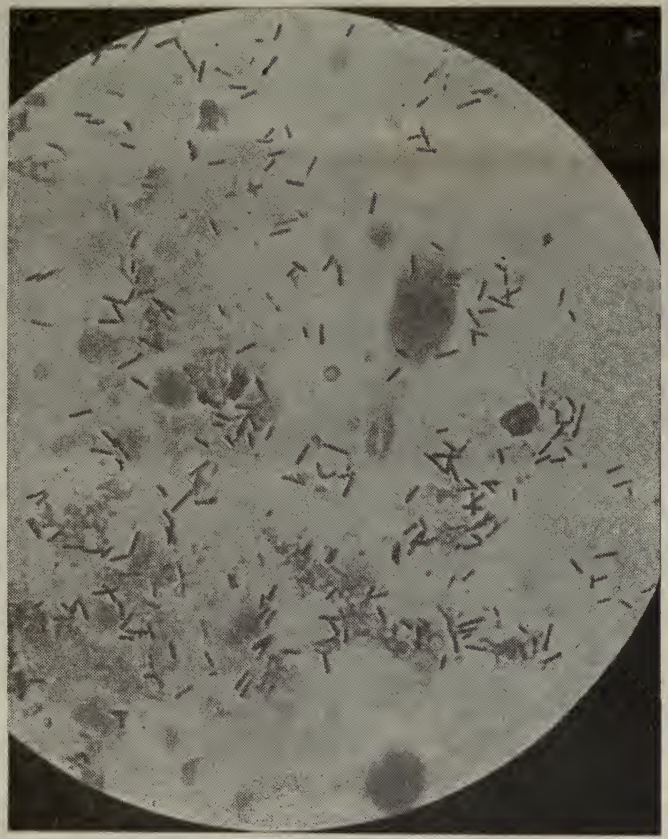

Fig. 5.

Culture from hepatic necrotic focus, Case 30, twenty-four hours liver piece broth ; Claudius stain. Obj : 6 m.m. Oc : 2. 55 c.m. x 400.

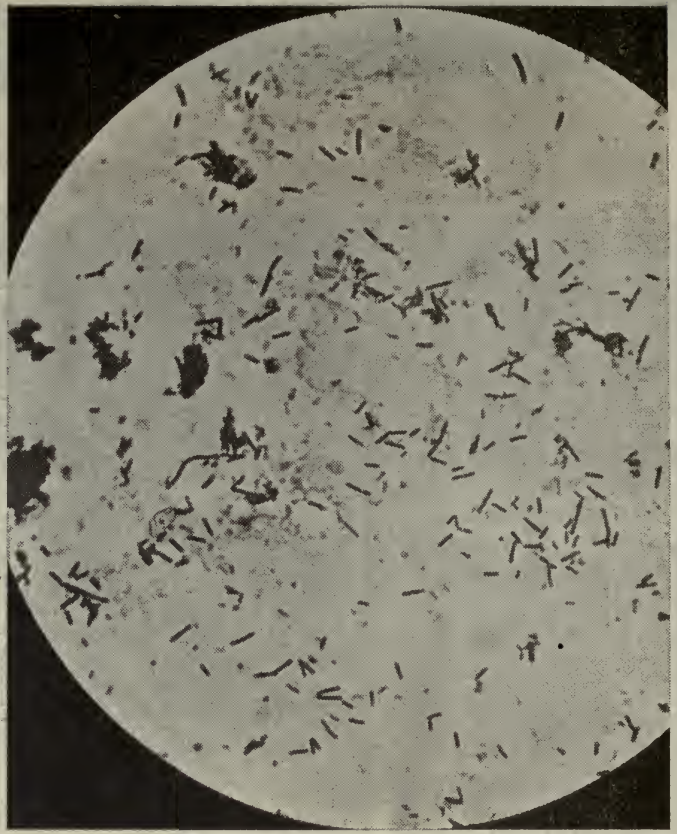

Fig. 6.

Twenty-four hours liver piece broth culture from subcutaneous exudate, guinea pig 147 ; Claudius stain. Obj. 6 m.m. Oc : 2. 55 c.m. x 400.

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